

Applicant : Lorin R. DeBonte et al.
Serial No. : 08/572,027
Filed : December 14, 1995
Page : 2 of 10

Attorney's Docket No.: 07148-032001
/ CGL99/0007US5A, A015-00505.0010

Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1. (previously presented) An isolated nucleic acid fragment comprising a sequence of at least about 20 nucleotides from a *Brassicaceae* or *Helianthus* delta-12 fatty acid desaturase gene having at least one mutation in a region of said desaturase gene encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said at least one mutation renders the product of said desaturase gene non-functional and wherein said sequence includes said at least one mutation.
2. (original) The nucleic acid fragment of claim 1, wherein said sequence comprises a full-length coding sequence of said gene.
3. (currently amended) The nucleic acid fragment of claim 1, wherein said ~~mutant desaturase gene~~ sequence encodes a microsomal gene product.
4. (canceled)
5. (currently amended) The nucleic acid fragment of claim 1, wherein said at least one mutation in said region of said desaturase gene introduces a non-conservative amino acid substitution in said motif.
6. (currently amended) The nucleic acid fragment of claim 5, wherein the wild-type sequence of said motif ~~comprises~~ has the sequence His-Glu-Cys-Gly-His (SEQ ID NO:9).
7. (previously presented) The nucleic acid fragment of claim 6, wherein said at least one mutation in said motif comprises a codon encoding Lys in place of the codon encoding Glu.

Applicant : Lorin R. DeBonte et al.
Serial No. : 08/572,027
Filed : December 14, 1995
Page : 3 of 10

Attorney's Docket No.: 07148-032001
/ CGL99/0007US5A, A015-00505.0010

8. (original) The nucleic acid fragment of claim 1, wherein said mutant desaturase gene is from a *Brassica napus* plant.

9. (canceled)

10. (currently amended) The nucleic acid fragment of claim 66, wherein said at least one mutation in said motif comprises a codon encoding His in place of the codon encoding Leu.

11.-26. (canceled)

27. (previously presented) An isolated nucleic acid fragment comprising a sequence of at least about 20 nucleotides from a *Brassicaceae* delta-15 fatty acid desaturase gene having at least one mutation in a region of said desaturase gene encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said at least one mutation renders the product of said desaturase gene non-functional and wherein said sequence includes said at least one mutation.

28. (original) The nucleic acid fragment of claim 27, wherein said sequence comprises a full-length coding sequence of said gene.

29. (currently amended) The nucleic acid fragment of claim 27, wherein said ~~mutant desaturase gene~~ sequence encodes a microsomal gene product.

30. (canceled)

31. (previously presented) The nucleic acid fragment of claim 27, wherein said at least one mutation introduces a non-conservative amino acid substitution in said region.

Applicant : Lorin R. DeBonte et al.
Serial No. : 08/572,027
Filed : December 14, 1995
Page : 4 of 10

Attorney's Docket No.: 07148-032001
/ CGL99/0007US5A, A015-00505.0010

32. (currently amended) The nucleic acid fragment of claim 31, wherein the wild-type amino acid sequence of said motif comprises the sequence His-Asp-Cys-Gly-His (SEQ ID NO:9).

33. (previously presented) The nucleic acid fragment of claim 32, wherein said at least one mutation comprises a codon encoding Lys in place of the codon encoding Asp.

34. (original) The nucleic acid fragment of claim 27, wherein said mutant desaturase gene is from a *Brassica napus* plant.

35. (currently amended) A *Brassicaceae* plant containing a full-length coding sequence of a delta-15 fatty acid desaturase gene having at least one mutation, wherein said at least one mutation is in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif and wherein said mutation renders the product of said desaturase gene non-functional.

36. (canceled)

37. (previously presented) The plant of claim 35, wherein said mutation confers a decreased level of α -linolenic acid in seeds of said plant.

38. (original) The plant of claim 35, wherein said mutant desaturase gene encodes a microsomal gene product.

39. (original) The plant of claim 35, wherein said at least one mutation comprises a non-conservative amino acid substitution in said region.

40. (currently amended) The plant of claim 39, wherein the wild-type amino acid sequence of said motif comprises the sequence His-Asp-Cys-Gly-His (SEQ ID NO:9).

Applicant : Lorin R. DeBonte et al.
Serial No. : 08/572,027
Filed : December 14, 1995
Page : 5 of 10

Attorney's Docket No.: 07148-032001
/ CGL99/0007US5A, A015-00505.0010

41. (previously presented) The plant of claim 40, wherein said at least one mutation comprises a codon encoding Lys in place of the codon encoding Asp.

42. (original) The plant of claim 35, wherein said mutant desaturase gene is from a *Brassica napus* plant.

43. (original) The plant of claim 35, wherein said plant is a *Brassica napus* plant.

44. (previously presented) A *Brassicaceae* plant containing:

- a) a full-length coding sequence from a delta-12 fatty acid desaturase gene having at least one mutation, said at least one delta-12 gene mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif; and
- b) a full-length coding sequence from a delta-15 fatty acid desaturase gene having at least one mutation, said at least one delta-15 gene mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif;

wherein said delta-12 gene mutation and said delta-15 gene mutation render the products of said delta-12 desaturase gene and said delta-15 desaturase gene, respectively, non-functional.

45. (previously presented) The plant of claim 44, wherein said mutant genes confer a decreased level of α -linolenic acid in seeds of said plant compared to α -linolenic acid levels in corresponding seeds lacking said mutant genes.

46. (currently amended) A *Brassicaceae* or *Helianthus* plant containing a full-length coding sequence of a delta-12 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a Tyr-Leu-Asn-Asn-Pro (SEQ ID NO:50) amino acid motif and wherein said mutation renders the product of said desaturase gene non-functional.

Applicant : Lorin R. DeBonte et al.
Serial No. : 08/572,027
Filed : December 14, 1995
Page : 6 of 10

Attorney's Docket No.: 07148-032001
/ CGL99/0007US5A, A015-00505.0010

47.-54. (canceled)

55. (currently amended) A method for producing a *Brassicaceae* or *Helianthus* plant line, comprising the steps of:

- a) inducing mutagenesis in cells of a starting variety of a *Brassicaceae* or *Helianthus* species;
- ~~(b)~~ b) obtaining one or more progeny plants from said cells;
- ~~(e)~~ c) identifying at least one of said progeny plants that contains a delta-12 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said mutation in said delta-12 gene mutation renders the product of said delta-12 desaturase gene non-functional; and
- ~~(d)~~ d) producing said plant line from said at least one progeny plant by self or cross-pollination, said plant line having said at least one mutation in said delta-12 gene mutation.

56. (original) The method of claim 55, wherein said plant line yields an oil having a stabilized linoleic acid content from about 2.0 % to about 12.0 %.

57. (currently amended) The method of claim 55, further comprising the steps of:
- e) inducing mutagenesis in cells of said plant line;
 - f) obtaining one or more progeny plants from said cells of said plant line;
 - g) identifying at least one of said plant line progeny plants from step f) that contains a delta-15 fatty acid desaturase gene having at least one mutation, wherein said at least one mutation in said delta-15 gene mutation is in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said delta-15 gene mutation renders the product of said delta-15 desaturase gene non-functional; and

Applicant : Lorin R. DeBonte et al.
Serial No. : 08/572,027
Filed : December 14, 1995
Page : 7 of 10

Attorney's Docket No.: 07148-032001
/ CGL99/0007US5A, A015-00505.0010

h) producing a second plant line from said at least one plant line progeny ~~plant by self or cross pollination identified in step g)~~, said second plant line having said at least one mutation in said delta-12 gene ~~mutation~~ and said at least one mutation in said delta-15 gene ~~mutation~~.

58. (original) The method of claim 55, wherein said starting variety is a *Brassica napus* variety.

59. (original) The method of claim 58, wherein said mutation is in a first form of delta-12 fatty acid desaturase.

60. (original) The method of claim 59, further comprising the step of crossing a plant of said plant line to a plant having a mutation in a second form of delta-12 fatty acid desaturase.

61. (original) The method of claim 60, wherein said second mutation is in a region other than a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif.

62. (currently amended) The method of claim 59, further comprising the steps of:

- e) inducing mutagenesis in cells of said plant line;
- f) obtaining one or more progeny plants from said cells of said plant line ~~cells~~;
- g) identifying at least one of said ~~plant line progeny~~ plants from step f) that contains a second delta-12 fatty acid desaturase gene having at least one mutation, said second gene mutation in a region other than a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif; and
- h) producing a second plant line from said at least one plant line progeny ~~plant by self or cross pollination identified in step g)~~, said second plant line having said first and second delta-12 gene mutations.

Applicant : Lorin R. DeBonte et al.
Serial No. : 08/572,027
Filed : December 14, 1995
Page : 8 of 10

Attorney's Docket No.: 07148-032001
/ CGL99/0007US5A, A015-00505.0010

63. (original) The method of claim 55, wherein said identifying step comprises a technique selected from the group consisting of: PCR, 3SR and direct polynucleotide sequencing.

64. (currently amended) A method for producing a *Brassicaceae* plant line, comprising the steps of:

- a) inducing mutagenesis in cells of a starting variety of a *Brassicaceae* or *Helianthus* species;
- b) obtaining one or more progeny plants from said cells;
- c) identifying at least one of said progeny plants that contains a delta-15 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said at least one mutation renders the product of said delta-15 desaturase gene non-functional; and
- d) producing said plant line from said at least one progeny plant by self or cross-pollination, said plant line having said mutation in said delta-15 gene ~~mutation~~.

65. (original) The method of claim 64, wherein said identifying step comprises a technique selected from the group consisting of: PCR, 3SR and direct polynucleotide sequencing.

66. (currently amended) An isolated nucleic acid fragment comprising a sequence of at least about 20 nucleotides from a *Brassicaceae* or *Helianthus* delta-12 fatty acid desaturase gene having at least one mutation in a region of said desaturase gene encoding a Tyr-Leu-Asn-Asn-Pro (SEQ ID NO:50) amino acid motif, wherein said at least one mutation renders the product of said desaturase gene non-functional and wherein said sequence includes said at least one mutation.

Applicant : Lorin R. DeBonte et al.
Serial No. : 08/572,027
Filed : December 14, 1995
Page : 9 of 10

Attorney's Docket No.: 07148-032001
/ CGL99/0007US5A, A015-00505.0010

67. (previously presented) A method for identifying a mutation in a *Brassicaceae* plant, comprising:

- a) providing a *Brassicaceae* plant having a decreased α -linolenic acid content as compared with a corresponding control *Brassicaceae* plant; and
- b) identifying at least one mutation in a delta-15 fatty acid desaturase gene of said plant, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said mutation renders the product of said delta-15 fatty acid desaturase gene non-functional.

68. (previously presented) The method of claim 67, wherein said identifying step comprises a technique selected from the group consisting of: PCR, 3SR and direct polynucleotide sequencing.

69. (previously presented) A method for identifying a mutation in a *Brassicaceae* or *Helianthus* plant, comprising:

- a) providing a *Brassicaceae* or *Helianthus* plant having an increased oleic acid content as compared with a corresponding control *Brassicaceae* or *Helianthus* plant; and
- b) identifying at least one mutation in a delta-12 fatty acid desaturase gene of said plant, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said mutation renders the product of said delta-12 fatty acid desaturase gene non-functional.

70. (previously presented) The method of claim 69, wherein said identifying step comprises a technique selected from the group consisting of: PCR, 3SR and direct polynucleotide sequencing.